potential upstroke. These data are the first report of FGF13 as regulator of Nav1.5 in heart and suggest that FGF13 isoform-specific regulation of cardiac Nav channels plays important physiological and pathophysiological roles.

2279-Pos Board B265

CaMKII Regulates Cardiac Sodium Channel Nav1.5 by Phosphorylation in the Loop Between Domain I and II

Anthony W. Herren, Nicole M. Ashpole, Kenneth S. Ginsburg, Donald M. Bers, Andy Hudmon.

The cardiac Na channel $Na_V1.5$ current (I_{Na}) is critical to cardiac excitability and altered I_{Na} gating has been implicated in genetic and acquired arrhythmias. Ca-calmodulin dependent protein kinase (CaMKII) is upregulated in heart failure and has been shown to cause I_{Na} gating changes that mimic a point mutant in humans associated with combined long QT and Brugada syndromes. We sought to identify the CaMKII phosphorylation target site on $Na_V1.5$ that mediates the CaMKII-induced alterations in I_{Na} gating. We analyzed CaMKII-dependent phosphorylation of $Na_V1.5$ intracellularly accessible sites using a series of GST-fusion constructs, immobilized peptide arrays, and soluble peptides. CaMKII phosphorylation sites were identified at S516 and S593/T594 in the intracellular $Na_V1.5$ loop between domains 1 and 2. Wild-type (WT) and phospho-mutant human Nav1.5 were co-expressed with CaMKIIδ_C-GFP in HEK293 cells and I_{Na} was recorded by whole-cell patch clamp under pipette conditions that acutely activated CaMKII (+1 μM free [Ca], + 1 μM Calmodulin). As observed in myocytes, CaMKII shifted WT I_{Na} availability to more negative membrane potentials and enhanced accumulation of I_{Na} into intermediate inactivation, but these effects were abolished by mutating either of the CaMKII sites to non-phosphorylatable alanine residues ($V_{1/2}$ of availability: WT −101.8 ± 4.5 (n=10); S593/T594AA −89.7 ± 3.5 (n=11) ; S516A −93.0 ± 5.5 (n=18)). Furthermore, the phospho-mutant channels mimicked WT I_{Na} treated with 1 μM AIP, a specific CaMKII inhibitor, and AIP had no effect on phospho-mutant I_{Na}. CaMKII activation had no effect on the $V_{1/2}$ of activation or I-V curve shape of any channel. CaMKII-dependent phosphorylation of $Na_V1.5$ at multiple sites (including Ser593/Thr594 and Ser516) appears to be required to evoke loss-of-function changes in gating that could contribute to acquired Brugada syndrome like effects in heart failure.

2280-Pos Board B266

Chlorobutanol, a Pharmaceutical Preservative and Sedative Hypnotic, Inhibits Brain Type Voltage Gated Sodium Channels

George Kracke, Alice Landrum.

Introduction- Chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol) is a widely used chemical preservative for injectable drugs, eye drops, mouth washes, and cosmetics. It is an analgesic, experimental general anesthetic, and sedative hypnotic in man. The mechanism of action of chlorobutanol is unclear. Our goal was to test whether chlorobutanol inhibits brain type voltage gated sodium channels.

Methods- Mammalian Na_v 1.2 voltage gated sodium channels were expressed in *Xenopus* oocytes by injecting the alpha subunit cRNA. Inward sodium currents were measured with two-electrode voltage clamp using standard depolarization protocols. The study was approved by our institutional animal care and use committee.

Results- Chlorobutanol was tested at concentrations ranging from 0.03 to 10 mM. Chlorobutanol reversibly inhibited closed, resting channels and inactivated channels in a concentration dependent manner. The voltage dependence of activation was shifted in the depolarizing direction. Fast inactivation voltage dependence was not affected. There was little state dependent block and no apparent use dependence.

Discussion and Conclusions- Chlorobutanol inhibited Na_v 1.2 channels at concentrations less than those used to preserve pharmaceuticals. Its use as a preservative in injectable methadone and local anesthetic solutions, and in eye drops likely adds an analgesic component to these solutions.

2281-Pos Board B267

Enhancement of Slow Inactivation of Voltage-Gated Na^+ Channels by Ranolazine

Ryoko Hirakawa, Lynda V. Liu, John C. Shryock, Luiz Belardinelli, Sridharan Rajamani.

Ranolazine is an anti-anginal drug that blocks cardiac ($Na_v1.5$) late Na^+ current (I_{Na}) at therapeutic concentrations (2-10 μM). Recent electrophysiological studies have shown that ranolazine also blocks skeletal muscle ($Na_v1.4$) and neuronal ($Na_v1.1$, $Na_v1.7$, $Na_v1.8$) I_{Na}. We investigated the effects of ranolazine on peak I_{Na} and on both sustained repetitive firing (SRF) and 0 mM Mg^{2+}- induced (0 $[Mg^{2+}]_O$) continuous high frequency firing of action potentials in cultured rat hippocampal neurons. Ranolazine caused a voltage (−60 mV) and frequency (10 Hz)-dependent inhibition of I_{Na} with IC_{50} values of 0.48 and 61.8 μM (n=4-6), respectively. Ranolazine (10 μM, n=4) did not shift the voltage-dependence of steady-state fast inactivation; however, it caused a 15-mV hyperpolarizing shift ($p<0.05$, compared to control, n=4) in the slow inactivation. Consistently, ranolazine (10 μM) reduced SRF only during a 4-sec burst but not with a 1-sec depolarization step (Fig.1). Furthermore, ranolazine (10 μM) reduced 0 $[Mg^{2+}]_O$ -induced high frequency firing of spontaneous action potentials from 0.78 to 0.45 Hz ($p<0.05$, n=5). Taken together, these data suggest that ranolazine suppresses the propagation and conduction of action potentials by preferentially interacting with Na^+ channels in the slow inactivated state.

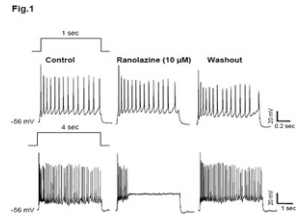

Fig. 1: Representative tracings of 1 and 4 sec sustained repetitive firing of action potentials in cultured hippocampal neurons in the absence (control) and presence of ranolazine (10 μM) followed by washout.

2282-Pos Board B268

Y1767C, a Novel *SCN5A* Mutation Induces a Persistent Sodium Current and Potentiates Ranolazine Inhibition of $Na_V1.5$ Channels

Hai Huang, Silvia G. Priori, Carlo Napolitano, Michael E. O'Leary, **Mohamed Chahine**.

Long QT syndrome type 3 (LQT3) has been traced to mutations of the cardiac Na channel ($Na_v1.5$) that produce persistent Na currents leading to delayed ventricular repolarization and Torsades de pointes. We performed mutational analyses of patients suffering from LQTS and characterized the biophysical properties of the mutations that we uncovered. One LQT3 patient carried a mutation in the *SCN5A* gene in which the cysteine was substituted for a highly conserved tyrosine (Y1767C) located near cytoplasmic entrance of the $Na_v1.5$ channel pore. The wild-type and mutant channels were transiently expressed in tsA201 cells, and the Na currents were recorded using the patch-clamp technique. The Y1767C channel produced a persistent Na current, more rapid inactivation, faster recovery from inactivation, and an increased window current. The persistent Na current of the Y1767C channel was blocked by ranolazine but not by many class I antiarrhythmic drugs. The incomplete inactivation, along with the persistent activation of Na channels caused by an overlap of voltage-dependent activation and inactivation, known as window currents, appeared to contribute to the LQTS phenotype in this patient. The blocking effect of ranolazine on the persistent Na current suggested that ranolazine may be an effective therapeutic for treating patients with this mutation. Our data also revealed the unique role for the Y1767 residue in inactivating and forming the intracellular pore of the $Na_v1.5$ Na channel.

2283-Pos Board B269

The Selective $Na_v1.8$ Sodium Channel Blocker A-803467 Affects Electrical Activity in Intracardiac Neurons, but not in Cardiomyocytes

Marieke W. Veldkamp, Carol A. Remme, Brendon Scicluna, Cees A. Schumacher, Rianne Wolswinkel, Antoni C.G. van Ginneken, Connie R. Bezzina, Arie O. Verkerk.

Background. Recently we observed differential myocardial expression of the brain-type sodium channel isoform *Scn10a* between two inbred mouse strains, both harboring the Scn5a-1798insD+/- mutation and displaying different severity of conduction disease. The functional role of *Scn10a* in the heart is as yet unknown, and we therefore investigated expression and channel activity of $Na_V1.8$ (encoded by *Scn10a*) in intracardiac neurons and myocardium of the murine heart.

Methods. Immunocytochemistry was performed using anti-$Na_V1.8$ antibody on mouse embryos and adult murine cardiac tissue sections. The effect of the $Na_V1.8$ blocker A-803467 (500 nM) on action potentials (AP's) and sodium current (I_{Na}) properties was assessed in isolated intracardiac neurons and ventricular myocytes.

Results. In embryonic and adult heart tissue sections, $Na_V1.8$ staining was observed at the epicardial surface, and within the myocardium in between cardiomyocytes. The $Na_V1.8$ blocker A-803467 had no effect on either mean I_{Na} density or I_{Na} kinetic properties in isolated myocytes, but clearly reduced I_{Na} density in intracardiac neurons (−344 ± 51 pA/pF versus control −448 ± 61; mean ± SEM, n=11). In addition, the slow component of the current decay (τ_{slow}) at −20 mV was accelerated in the presence of A-803467 (2.8 ± 0.3 ms versus control 3.4 ± 0.4 ms; mean ± SEM, n=5) and $V_{1/2}$ of voltage-dependent inactivation was shifted by −9.6 mV (−73.6 ± 2.0 mV versus control −64.0 ± 1.6 mV; mean ± SEM, n=5). This is consistent with a reduction in slowly inactivating brain-type sodium current with depolarized voltage-dependent inactivation. In AP measurements A-803467 did not affect cardiomyocyte upstroke velocity, but reduced AP firing frequency in intracardiac neurons by 50%.